Hematology and Biochemistry Reference Values for Ontario Swine

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ABSTRACT

The purpose of this study was to establish blood reference values for Ontario swine from various age groups. Weanling pigs, feeder pigs, gilts and sows on 11 randomly selected swine farms were sampled using the orbital sinus bleeding technique. Routine hematological and biochemical determinations were performed using whole blood and serum. For the variables examined in each age group the means, the standard deviations and the 95% upper and lower limits were calculated.

Key words: Swine, blood biochemistry, hematology, reference values, age groups.

RÉSUMÉ

Cette étude visait à déterminer des paramètres sanguins de référence, pour les porcs de l'Ontario. Les auteurs utilisèrent à cette fin des porcelets récemment sevrés, des porcs à l'engrais, ainsi que des truies jeunes et adultes, qu'ils choisirent dans 11 porcheries et dont ils prélevèrent des échantillons de sang, avec et sans anticoagulant, du sinus orbital. Ils déterminèrent les paramètres hématologiques et biochimiques conventionnels, en utilisant le sang entier et le sérum, et, pour chacun des groupes d'âge, ils calculèrent la moyenne, la déviation standard, ainsi que la limite de confiance à 95%, en plus ou en moins.

Mots clés: porcs, biochimie du sang, hématologie, valeurs de référence, groupes d'âge.

INTRODUCTION

The establishment of reference values is an important basis for clinical interpretation of laboratory data (1,2). The hematological and biochemical parameters of swine are influenced by a wide range of environmental and physiological factors including diet, age, sex and housing (3,4). Advances in technology over the past decade have dramatically changed Ontario swine production and have led to improvements in biochemical laboratory techniques. The trend in swine production is to large, more intensive confinement systems. The number of pork producers in Ontario has been reduced by one-third over the past decade and yet the number of pigs marketed per year has increased by more than one million (5). Clinical laboratories have introduced automated and computerized systems with changes in methodology which have improved the overall precision and accuracy of biochemical measurements and markedly reduced costs per test. Furthermore, physiological knowledge concerning many of the important enzyme systems has greatly expanded over the past few years. Hematological and biochemical reference values reflecting these latest advances are required to accommodate the many changes occurring in pig farming and laboratory technology and to facilitate the expansion of a scientific data base.

MATERIALS AND METHODS

Swine farms were randomly selected from the Ontario Pork Producers Marketing Board files. From this initial list 11 farms were selected for the study according to the following criteria:

a) the farm was located in one of the seven largest pork producing counties of Ontario (Huron, Perth, Waterloo, Wellington, Oxford, Middlesex and Lambton).
b) the farm shipped more than 1000 market hogs the previous year.
c) the producer agreed to participate in the study.

Wherever possible 40 animals were chosen for blood sampling on each farm. Ten pigs were randomly selected from four production groups (weaner pigs, feeder pigs, gilts and mature sows). Blood was collected from these animals using the orbital sinus bleeding technique (6) into 10 mL vials containing K EDTA, heparin, or no anticoagulant for whole blood, plasma and serum respectively. Smears were made for the samples containing EDTA and plasma and serum were separated by centrifugation using silicone separators. Serum and plasma were held at 4° C and samples containing distinct hemolysis were discarded. Most assays were completed within 24 hours.

Standard hematology techniques were used for determination of leukocytes, erythrocytes, hematocrit, hemoglobin and erythrocyte indices (Coulter Model S®, Coulter Electronics of Canada, Ltd., Burlington, Ontario). A KDA biochemistry instrument and packaged reagents (American Monitor Corporation, Mississauga, Ontario) were used for determination of serum biochemistry variables. The calcium assay is based upon a colour product formed when calcium in an alkaline medium reacts with o-cresolphthalein complexone. The

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phosphorus assay depends upon formation of phosphomolybdenum blue. Total protein and albumin assay utilize biuret and bromcresol green reagents respectively. The urea assay uses o-phthalaldehyde, isoin-doline and methoxyguinolone to form a coloured product. Creatinine assay uses alkaline picrate; glucose is determined using an initial glucose oxidase reaction; cholesterol is determined enzymatically and includes free and esterified cholesterol. Total and conjugated bilirubin methodology is based upon diazotization with unconjugated bilirubin calculated by difference. Serum assays are incubated and read at 37° C. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity correlate with the disappearance of NADH in double enzymatic reactions. Alkaline phosphatase utilizes p-nitrophenylphosphate as a substrate to form p-nitrophenol which is quantitated photometrically. A kinetic "reverse reaction" modified Rosalki method is used for creatine kinase and an amyloclastic technique for amylase activity.

The blood glutathione peroxidase (GSH-Px) activity was determined by measuring the rate of glutathione oxidation by t-butyl hydroperoxide as catalyzed by the GSH-Px present in a hemolysate (7). The method was modified to be performed on an automated spectrometer (LKB 8600 Reaction Rate Analyzer, LKB-Productes AB, S-16/25 Bromo, Sweden).

Reference values obtained were examined by age group (2) and the reference limits were calculated (2). After outliers had been eliminated using Dixon's r statistics (1), the variable means and 95% lower and upper confidence limits were calculated if the original data was from or could be transformed to a normal distribution using three forms of transformation (1). Otherwise, nonparametric percentile estimates were determined (1). Within each variable, age group means were compared using a Duncan's test (8).

RESULTS

In Tables I and II the lower and upper limit estimates are listed for the 95% range according to age group for each population variable examined. For the variables examined in each age group the following information is given in Tables III and IV: the sample size (n), transformations used (d), the mean ($\bar{x}$), the standard deviation (SD) and those variable means which were not significantly different according to age group.

For many variables the age group values did not have a normal (gaussian) distribution. The three transformations (logarithmic, square root and inverse) examined were more frequently successful in normalizing the distributions of these variables for the biochemistry than for the hematology data. Age group differences were present for many variables (Tables III and IV).

DISCUSSION

Hematology and biochemistry reference values have been determined for four age groups of pigs from 11 Ontario farms. Reference values are required by clinicians or researchers as a partial basis for interpretation of laboratory results from an individual pig or a group of pigs from a production unit. Reference limits were defined statistically by 95% confidence limits.

The interpretation of biochemical and hematological data from individual animals is limited by the wide animal to animal variation which occurs in normal populations. Age is an important source of variation for some of the parameters. The total serum protein, the mean corpuscular hemoglobin (MCH) and the mean corpuscular hemoglobin concentration (MCHC) increased with the age of the pigs (Table III and IV). The leukocyte count, the serum phosphorous and cholesterol concentrations and the alkaline phosphatase activity were lower in older pigs (Table III and IV). Other factors, such as sex, genetics, rate of growth, diet and feeding methods were not examined in this study, but have been shown to influence certain biochemical parameters (4). The stage of gestation can also effect the serum biochemical profile of the sow. Serum albumin, total protein, total bilirubin concentrations are lower in early pregnancy, whereas aspartate amino transferase and lactic dehydrogenase are higher in early pregnancy (9). The gilts sampled in this study were generally females that had never been bred, however the sows represented older animals in early and mid-gestation. Therefore, age as well as pregnancy status may be partly responsible for the variations in biochemical and hematological values observed between gilts and sows in this study.

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TABLE I. Porcine Hematology Reference Intervals

Variable	Units	Weaner Pigs	Feeder Pigs	Gilts	Sows
B-Hemoglobin	g/L	90 - 140	100 - 150	120 - 170	100 - 170
B-Hematocrit	L/L	0.26 - 0.41	0.29 - 0.42	0.33 - 0.45	0.29 - 0.46
B-Erythrocytes	$\times 10^{12}/L$	5.3 - 8.0	5.7 - 8.3	5.9 - 8.7	5.1 - 8.0
(B)Erc-MCV	fL	42 - 62	44 - 56	48 - 62	52 - 63
(B)Erc-MCH	pg	14 - 21	15 - 20	17 - 22	18 - 22
(B)Erc-MCHC	g/L	320 - 360	320 - 380	340 - 380	340 - 380
B-Leukocytes	$\times 10^{9}/L$	8.7 - 37.9	11.6 - 32.9	11.2 - 28.8	10.6 - 24.0
B-Neutrophils					
Segmented	$\times 10^{9}/L$	2.5 - 23.0	0.3 - 15.2	1.4 - 11.6	1.9 - 10.1
	%	16.6 - 73.1	4.4 - 62.1	11.1 - 53.7	15.1 - 59.5
Bands	$\times 10^{9}/L$	0.0 - 3.1	0.0 - 1.9	0.0 - 0.7	0.0 - 0.6
	%	0.0 - 13.0	0.0 - 8.0	0.0 - 2.8	0.0 - 3.3
B-Lymphocytes	$\times 10^{9}/L$	2.2 - 16	3.6 - 18.5	3.9 - 16.8	3.7 - 14.7
	%	12.5 - 70.1	21.2 - 78.0	30.4 - 74.5	25.5 - 71.1
B-Monocytes	$\times 10^{9}/L$	0.001 - 5	0.0 - 4.9	0.0 - 4.0	0.0 - 2.4
	%	0.0 - 17.0	0.1 - 20.1	0.2 - 20.8	1.0 - 14.0
B-Eosinophils	$\times 10^{9}/L$	0.0 - 1.8	0.0 - 2.5	0.0 - 3.3	0.0 - 2.4
	%	0.0 - 6.0	0.0 - 11.1	0.0 - 16.9	1.0 - 13.0
B-Basophils	$\times 10^{9}/L$	0.0 - 0.5	0.0 - 0.7	0.0 - 0.7	0.0 - 0.5
	%	0.0 - 2.0	0.0 - 3.6	0.0 - 3.8	0.0 - 3.0
B-Rubricytes	$\times 10^{9}/L$	0.0 - 0.2	0.0 - 0.3	0.0 - 0.3	0.0 - 0.2
	%	0.0 - 1.0	0.0 - 1.0	0.0 - 1.8	0.0 - 1.0
Disintegrated	$\times 10^{9}/L$	0.0 - 1.7	0.0 - 3.3	0.0 - 1.7	0.0 - 1.5
	%	0.0 - 7.4	0.0 - 14.2	0.0 - 5.9	0.0 - 9.3

TABLE II. Porcine Biochemistry Reference Intervals

Variable	Units	Weaner Pigs	Feeder Pigs	Gilts	Sows
S-Calcium	mmol/L	2.02 - 3.21	2.16 - 2.92	2.22 - 2.91	1.98 - 2.87
S-Phosphorous	mmol/L	1.46 - 3.45	2.25 - 3.44	1.88 - 2.78	1.49 - 2.76
S-Urea Nitrogen	mmol/L	2.90 - 8.89	2.57 - 8.57	1.70 - 9.60	2.10 - 8.50
S-Creatinine	mmol/L	67 - 172	77 - 165	106 - 225	110 - 260
S-Glucose	mmol/L	3.5 - 7.4	4.0 - 8.1	3.0 - 6.3	2.9 - 5.9
S-Cholesterol	mmol/L	1.06 - 3.32	1.37 - 3.18	1.37 - 2.70	1.23 - 2.74
S-Bilirubin	mmol/L	0.9 - 3.4	0.0 - 3.4	0.0 - 3.0	0.0 - 3.4
S-Conj. Bilirubin	mmol/L	0.0 - 3.4	0.0 - 1.7	0.1 - 1.7	0.0 - 1.7
S-Free Bilirubin	mmol/L	0.0 - 3.4	0.0 - 3.4	0.0 - 3.4	0.0 - 3.4
S-Iron	mmol/L	3 - 38	39 - 43	11 - 35	9 - 34
S-UIBC	mmol/L	43 - 96	48 - 101	57 - 106	54 - 99
S-AST	U/L	21 - 94	16 - 67	12 - 65	36 - 272
S-ALT	U/L	8 - 46	15 - 46	17 - 56	19 - 76
S-Alk. Phos.	U/L	142 - 891	180 - 813	115 - 434	36 - 272
S-CK	U/L	81 - 1,586	61 - 1,251	89 - 886	120 - 10,990
S-Amylase	U/L	528 - 2,616	913 - 4,626	643 - 4,668	433 - 2,170
S-Protein	g/L	44 - 74	52 - 83	65 - 81	65 - 90
S-Albumin	g/L	19 - 39	19 - 42	32 - 44	31 - 43
S-A/G	g/g	0.5 - 2.2	0.4 - 1.5	0.7 - 1.5	0.6 - 1.3
B-GSHPx	μ/gHb	30 - 137	40 - 141	44 - 127	48 - 135

TABLE III. Porcine Hematology Reference Values

Variable	Units	Weaner Pigs				Feeder Pigs				Gilts				Sows			
		n	d	$\bar{x}$	SD	n	d	$\bar{x}$	SD	n	d	$\bar{x}$	SD	n	d	$\bar{x}$	SD
B-Hemoglobin	g/L	109	G	110	10	93	G	120	10	89	G	140	10	108	G	130	10
B-Hematocrit	L/L	109	G	0.33	0.0	93	G	0.36	0.0	89	G	0.39	0.03	108	G	0.37	0.0
B-Erythrocytes	$X10^{12}/L$	108	$\frac{1}{x}$	6.5[a]	0.6	93	G	7.0	0.6	90	G	7.3	0.6	108	G	6.5[a]	0.7
(B)Erc-MCV	fL	109	G	52	5	93	NP	51	3	90	$\frac{1}{x}$	54	3	108	NP	57	3
(B)Erc-MCH	pg	109	G	17[a]	2	92	G	18[a]	1	87	G	19	1	110	G	20	1
(B)Erc-MCHC	g/L	109	NP	340	10	93	G	350	10	90	G	360[a]	10	110	G	360[a]	10
B-Leukocytes	$X10^{9}/L$	109	G	23.3[a]	26.7	92	G	22.2[a]	4.9	90	G	20.0[b]	4.0	108	NP	16.3[b]	3.2
B-Neutrophils																	
Segmented	$X10^{9}/L$	105	$\sqrt{x}$	10.7	4.8	91	G	7.4[b]	3.6	86	G	6.5[ab]	2.4	105	G	6.0[a]	1.9
	%	106	G	44.9	13.0	98	G	33.2[a]	13.3	88	G	32.4[a]	9.7	109	G	37.3	10.2
Bands	$X10^{9}/L$	103	NP	0.7	0.8	90	NP	0.4	0.5	85	NP	0.1[a]	0.2	105	NP	0.1[a]	0.2
	%	106	NP	3.2	3.3	98	NP	1.8	2.2	86	NP	0.7[a]	0.9	107	NP	0.8[a]	1.1
B-Lymphocytes	$X10^{9}/L$	104	G	9.2	3.2	92	G	11.1[a]	3.4	86	G	10.4[a]	3.0	105	Logχ	7.8	2.3
	%	106	G	41.3	13.3	98	G	49.8[a]	13.2	88	G	52.4[b]	10.1	109	G	48.3[ab]	10.5
B-Monocytes	$X10^{9}/L$	105	$\sqrt{x}$	1.5	1.3	92	$\sqrt{x}$	1.5	1.2	85	Logχ	1.2	0.9	105	Logχ	0.8	0.5
	%	105	NP	6.2[ab]	4.6	98	$\sqrt{x}$	6.7[b]	4.6	87	NP	6.3[ab]	4.5	109	NP	5.2[a]	3.4
B-Eosinophils	$X10^{9}/L$	105	NP	0.4	0.5	92	$\sqrt{x}$	0.8	0.6	86	$\sqrt{x}$	1.1	0.8	106	Logχ	0.8	0.6
	%	106	NP	1.8	1.7	98	NP	3.8	2.7	88	$\sqrt{x}$	5.4[a]	4.0	109	NP	5.1[a]	2.9
B-Basophils	$X10^{9}/L$	104	NP	0.1	0.1	91	NP	0.1	0.2	85	NP	0.2	0.2	107	NP	0.1	0.2
	%	106	NP	0.3	0.6	97	NP	0.6[a]	1.0	87	NP	0.8[a]	1.0	109	NP	0.7[a]	1.0
B-Rubricytes	$X10^{9}/L$	104	NP	0.01	0.05	91	NP	0.02	0.07	86	NP	0.01	0.06	106	NP	0.01	0.04
	%	105	NP	0.05	0.20	98	NP	0.09	0.30	88	NP	0.06	0.30	109	NP	0.08	0.30
B-Disintegrated	$X10^{9}/L$	102	NP	0.30	0.50	90	NP	0.80	0.80	85	NP	0.30	0.50	104	NP	0.30	0.40
	%	103	NP	1.40[ab]	2.20	96	NP	3.40	3.70	83	NP	0.90[a]	1.50	106	NP	2.00[b]	2.40

*Means with similar superscripts are not significantly different ($p < 0.01$)

hematological profiles performed on a random sampling of animals within a herd to evaluate the subclinical health of the herd has been proposed as a valuable tool in modern preventive medicine (10). Several of the biochemical and hematological variables are strongly influenced by chronic disease and nutritional deficiencies (3,4,11,12, 13). The detection of trace element deficiencies can be facilitated by measuring the activity of dependent enzyme systems (13). The response in activity of a metalloenzyme to the dietary supplementation of a specific trace element has been suggested as being a very effective method of diagnosing trace element deficiency problems (12). Enzymes, such as glutathione peroxidase (GSH-Px) and alkaline phosphatase (Alk. Phos.) have been used as diagnostic aids in diagnosing selenium (14,15) and zinc deficiencies (16), respectively. Similarly low dietary levels of phosphorus have resulted in a decrease in alkaline phosphatase, experimentally (17).

In summary, the data presented in this paper provides a baseline for interpreting hematological and bio-

TABLE IV. Porcine Biochemistry Reference Values

		Weaner Pigs				Feeder Pigs				Gilts				Sows			
Variable	Units	n	d	$\bar{x}$	SD	n	d	$\bar{x}$	SD	n	d	$\bar{x}$	SD	n	d	$\bar{x}$	SD
S-Calcium	mmol/L	104	G	2.61^{b}	0.27	93	G	2.54^{a}	0.18	82	G	2.57^{ab}	0.15	102	NP	2.55^{a}	0.20
S-Phosphorous	mmol/L	104	NP	2.75^{a}	0.45	93	$\frac{1}{x}$	2.75^{a}	0.27	83	G	2.33	0.21	102	$\frac{1}{x}$	1.97	0.28
S-Urea Nitrogen	mmol/L	104	Logχ	5.30^{a}	1.30	93	$\sqrt{x}$	5.30^{a}	1.40	83	$\sqrt{x}$	5.60^{a}	1.80	102	G	5.30^{a}	1.50
S-Creatinine	mmol/L	104	NP	102	22	92	NP	126	19	83	$\frac{1}{x}$	166^{a}	27	102	$\frac{1}{x}$	160^{a}	32
S-Glucose	mmol/L	101	G	5.4^{b}	0.9	91	$\frac{1}{x}$	5.5^{b}	1.0	80	G	4.6^{a}	0.7	102	G	4.4^{a}	0.7
S-Cholesterol	mmol/L	103	G	2.19^{b}	0.52	93	G	2.27^{b}	0.41	83	G	2.03^{a}	0.31	100	G	1.99^{a}	0.35
S-Bilirubin	mmol/L	100	NP	2.0	0.7	93	NP	1.7^{a}	0.9	81	NP	1.7^{a}	0.5	101	NP	1.7^{a}	0.9
S-Conj. Bilirubin	mmol/L	97	NP	0.6^{a}	0.9	93	NP	0.4^{a}	0.7	78	NP	0.6^{a}	0.8	94	NP	0.6^{a}	0.8
S-Free Bilirubin	mmol/L	91	NP	1.6	0.8	84	NP	1.2^{a}	1.0	69	NP	1.2^{a}	0.9	86	NP	1.1^{a}	1.0
S-Iron	mmol/L	104	G	21^{a}	8	93	x	22^{ab}	9	88	G	23^{b}	6	102	G	22^{ab}	6
S-UIBC	mmol/L	104	G	70	12	93	G	75^{a}	12	88	G	86	11	102	G	76^{a}	10
S-AST	U/L	101	$\frac{1}{x}$	37^{a}	12	91	Logχ	35^{a}	12	83	NP	28	13	102	Logχ	24	9
S-ALT	U/L	102	G	27^{a}	9	93	$\sqrt{x}$	29^{a}	7	81	Logχ	32^{b}	9	102	$\frac{1}{x}$	33^{b}	11
S-Alk. Phos.	U/L	100	G	517	172	93	Logχ	406	141	83	Logχ	234	73	100	Logχ	110	54
S-CK	U/L	99	$\frac{1}{x}$	189	100	83	Logχ	353^{a}	272	73	Logχ	321^{a}	182	98	$\frac{1}{x}$	298^{a}	164
S-Amylase	U/L	98	G	1572	480	93	NP	2027^{a}	913	83	NP	1978^{a}	987	100	NP	1831^{a}	684
S-Protein	g/L	104	$\frac{1}{x}$	56	7	93	G	68	7	83	$\frac{1}{x}$	72	4	102	G	77	6
S-Albumin	g/L	104	Logχ	27	5	93	NP	31	6	83	NP	38^{a}	3	102	NP	37^{a}	3
S-A/G	g/g	104	NP	1^{b}	0.4	93	x	0.9^{a}	0.2	83	NP	1.1^{b}	0.2	102	NP	0.9^{a}	0.2
B-GSHPx	μ/gHb	109	G	83^{a}	25	93	G	90^{b}	23	90	G	85^{ab}	19	110	G	91^{b}	20

*Means with similar superscripts are not significantly different ($p < 0.01$)

chemical results from individual sick pigs and from herds with suspected subclinical disease or borderline nutritional deficiencies. However, the usefulness of reference values are restricted by biological variations between animals and by analytical differences between laboratories. Herd biochemical profiles have been only rarely used in swine veterinary practice and for this reason future studies involving problem herds are warranted in order to assess the value of this diagnostic technique.

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